Load signals assist the generation of movement dependent reflex reversal in the femur-tibia joint of stick insects

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Number of figures:3

Number of table:0

Number of pages:17

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Keywords: locomotion, campaniform sensilla, load, active reaction, stick insect, reflex reversal

Short title: Interaction between movement and load feedback
Abstract

Reinforcement of movement is an important mechanism by which sensory feedback contributes to motor control for walking. We investigate how sensory signals from movement and load sensors interact in controlling the motor output of the stick insect femur-tibia (FT-) joint. In stick insects, flexion signals from the femoral chordotonal organ (fCO) at the FT-joint and load signals from the femoral campaniform sensilla (fCS) are known to individually reinforce stance phase motor output of the FT-joint by promoting flexor and inhibiting extensor motoneuron activity. We quantitatively compared the time course of inactivation in extensor tibiae motoneurons in response to selective stimulation of fCS and fCO. Stimulation of either sensor effects extensor activity in a qualitatively similar manner but with a significantly different time course and frequency of occurrence. Inactivation of extensor motoneurons due to fCS stimulation was more reliable but more than three-fold slower as compared to the extensor inactivation in response to flexion signals from the fCO. In contrast, simultaneous stimulation of both sense organs produced inactivation in motoneurons with a time course typical for fCO stimulation alone, but with a frequency of occurrence characteristic for fCS stimulation. This increase in probability of occurrence was also accompanied by a delayed re-activation of the extensor motoneurons. Our results indicate for the first time that fCS load signals from the leg affect the processing of movement related feedback from the fCO in controlling motor output.
Introduction

During walking, proprioceptive information from the limbs continuously supplies the neural networks in the central nervous system with information about leg position and movement as well as forces generated in each leg (Grillner, 1981; Orlovsky et al., 1999; Pearson, 2004). In order to generate the basic stepping pattern and to adapt motor output to changing substrate conditions, sensory feedback from movement and force sensors in vertebrates and invertebrates regulates the magnitude of the motor output and initiates the transitions between the stance and the swing phase of the walking pattern (summaries Büschges 2005; Pearson 1993; 2000). One of the mechanisms involved is “reinforcement” of the ongoing motor output by sensory feedback. For example, in a stick insect leg during locomotion, flexion signals from the femur-tibia (FT-) joint enhance ongoing flexor activity while at the same time inhibiting extensor activity (Bässler, 1986, 1988). This motor response constitutes a reflex reversal, since the motor reflex in response to flexion of the tibia is reversed compared to the reflex observed in a resting animal. Due to this reflex reversal, flexion signals from the FT-joint can augment the generation of flexor motoneuron activity during the stance phase. As the flexion of the FT-joint increases, the flexor motoneuron activity terminates and the extensor activity resumes (Bässler, 1986). This sequence of motor responses to flexion signals from the FT-joint that always upon its generation affects flexor and extensor motoneurons simultaneously was originally called the “active reaction”, as it only occurs when the locomotor system of the stick insect is active (Bässler, 1986, 1988). Furthermore, sensory signals reporting loading of the leg in stick insects increases stance phase motor activity (Bässler, 1977; Wendler, 1964; Akay et al. 2001, 2004). Similar influences of movement and load sensors are also known in other walking systems such as the crayfish and the cat (DiCaprio & Clarac, 1981; Pearson & Collins, 1993; Whelan et al. 1995; for review see Büschges & ElManira, 1998; Clarac et al. 2000) and some information is available on the underlying neural pathways (reviews in Bässler & Büschges, 1998; Pearson, 2000; Zill et al.
2004). However, at present it is unknown whether and how the processing of movement and load signals may interact with each other in the generation of tibial motoneuron activity when the locomotor system is active. In the stick insect, both sensory modalities have been shown to interact at rest in vivo (Schmitz & Stein, 2000). However, in reduced preparations stimulation of one leg sensor alone was not sufficient to reliably elicit the appropriate flexor motor output. For example, in the femur-tibia joint control system, the incidence of the “active reaction” during fCO flexion signals is variable, ranging from as low as 25% to almost 100% in individual preparations (Bässler, 1986; 1988).

We investigated the interaction of movement and load in the stick insect femur-tibia (FT-) joint for which reinforcement of movement and load has been shown when the locomotor system is active (Akay et al. 2001; Bässler, 1988). By combining selective mechanical stimulation of movement and load sensors on the middle leg we show for the first time, that load and movement information processing interact significantly. Load signals from the leg assist the effectiveness of movement related feedback by priming the premotor network of the femur-tibia joint for generating movement dependent reflex reversal.

**Methods**

The experiments were performed using adult female stick insects (*Carausius morosus*) from our breeding colony at the University of Cologne. All experiments took place under dimmed daylight conditions and at room temperature (20°-22°). The experiments were carried out under two different conditions: in resting animals (inactive state) and in active animals (activated by touching the abdomen with a paint brush). In Bässler (1988) and Bässler and Büschges (1998) inactive and active behavioral states have been defined and their properties have been described in detail. Before fixing the animal on a platform, the sensory hairs of the left middle leg femur were shaved with a razor blade and the trochanteral campaniform
sensilla (trCS) were destroyed with a fine insect pin (Schmitz, 1993). The animals with only the left middle leg attached were glued dorsal side up on a foam platform with dental cement (Protemp II, ESPE). The left middle leg was then fixed on the platform perpendicular to the body axis and the femur-tibia joint (FT-joint) was set to approximately 120° and embedded in dental cement. A basin was built around the femur with Vaseline and filled with WD-saline (Weidler & Diecke, 1969). A cut along the midline of the meso- and metathoracic tergum were made and both sides were folded back and fixed with insect pins. The thorax was filled with WD-saline and the gut, fat, and connective tissue were removed to expose the ventral nerve cord. Care was taken to leave as much of the trachea system intact as possible. All lateral leg nerves were cut except for the innervation of the femoral chordotonal organ (fCO) and the femoral campaniform sensillae (fCS) and the connective nerves. The activity of the slow and the fast extensor tibiae motoneurons (SETi and FETi respectively) and the common inhibitor 1 (CI1) was monitored by extracellular recordings with hook electrodes (Schmitz et al., 1991, custom amplifier from the electronics workshop of the Dept of Animal Physiology, Zool. Inst., University of Cologne) either from lateral nerve nl3 or from the femoral nerve F2. To stimulate the femoral chordotonal organ (fCO), the femur of the left middle leg was opened from the dorsal side and the fCO apodeme and the F2 nerve exposed. The fCO apodeme was fixed to the clamp of an electromechanical puller (Hofmann et al. 1985). Movement of the tibia was mimicked by elongating or relaxing the fCO apodeme with trapezoidal waveforms within a range corresponding to an approximately 60° FT-joint angle change (Weiland et al. 1986). fCS were stimulated by means of a low-voltage piezo-electrical element (PI Physic), applying pressure to the proximal femoral cuticle close to the fCS (Akay et al. 2001). While this stimulation procedure does not mimic natural stimuli of the fCS, it effectively activates the fCS afferents as previously shown by Akay et al. (2001). To perform intracellular recordings from the neuropilar processes of extensor tibiae motoneurons, the mesothoracic ganglion was fixed on a wax-coated platform with fine cactus needles and
treated according to established procedures (Büsches, 1989). While the extensor tibiae muscle in orthopteran insects is only innervated by two excitatory motoneurons (Bässler & Storrer, 1980; Field & Burrows, 1982), the flexor tibiae muscle is innervated by at least 14 motoneurons (Debordt & Bässler 1990; Field & Burrows 1982). Due to the simpler motor supply to the extensor system we focused in our study on the analysis of synaptic inputs converging onto the extensor tibiae motoneurons. For the purpose of our study this was sufficient, because identification of the “active reaction” can be made by recording extensor motoneuron activity only. Bässler (1988) has shown that flexor motoneurons always generate alternating activity compared the extensor, i.e. they are always depolarized and activated during the first part of the active reaction and strongly hyperpolarized during the second part. It is thus possible to identify an active reaction on the basis of the extensor motor neuron activity only.

Thin-walled sharp microelectrodes were used for intracellular recordings in bridge and discontinuous current clamp (DCC) mode. The electrodes had a resistance of 15-25MΩ when filled with 0.1M KCl/3M KAc or 0.05M KCl/2M KAc. Tibial motoneurons were identified by a one-to-one correlation of their action potentials with the spikes in the F2 recording. Extracellular and intracellular recordings were stored on a DAT-Recorder (SONY, PC 116). Analog-to-digital conversion was performed (sampling rate: 12.5kHz for intracellular and extracellular recordings) on a CED 1401plus interface (Cambridge Electronic). The recordings were analyzed with Spike2 software (Versions 3.13-4.03). Statistical evaluation of data and plotting of the graphs was carried out with Excel 97. In the text, “N” gives the number of experiments and “n” gives the sample size. Differences in the means of samples were tested by using the Student’s t-test (Excel 97). In addition, binomial distributions were compared with the Chi-square test. Means were regarded as significantly different with p<0.05.
Results

When the stick insect walking system is in the active behavioral state, signals from at least two sense organs measuring different parameters provide inhibition to the extensor motoneurons and excitation to the flexor motoneurons. These sense organs are the fCO that signals movement of the FT-joint and the fCS that responds to force on the leg. Flexion of the FT-joint measured by the fCO can elicit the so-called “active reaction” (Bässler, 1988) in tibial motoneurons (Fig. 1A, i). The most prominent aspect of this response is a reflex reversal upon flexion signals from the fCO: flexor activity is initiated and extensor activity is terminated. Extensor motoneurons are re-activated (the second part of the “active reaction”; Bässler, 1986) during the interval when increasing flexion of the FT-joint is signaled by the fCO (Fig.1A, i). During fCO stimulation, the latency in termination of the extensor activity averaged 0.12s (SE (standard error): 0.02; N=7, n=29), evaluated between the start of the fCO stimulus and the last action potential in either SETi or FETi. Recommencement of extensor activity with fCO stimulation was occurring on average 0.32s (SE:0.02; n=29; p<0.0001) after the start of fCO elongation well before the end of the stimulus. Furthermore, load signals from the fCS, elicited by application of pressure to the cuticle on the posterior side of the proximal femur (see also Akay et al. 2001) also caused inactivation of extensor tibiae motoneurons similar to the first phase of the active reaction (Fig. 1A, ii), however extensor activity did not recommence before the end of the load stimulus. fCS stimulation produced a slower inactivation of extensor activity with a longer latency, which was on average 0.38s (SE:0.03; N=7, n=51) and the latency of extensor activity recommencement with fCS stimulation was 0.54s (SE:0.03; N=7, n=48; p=0.009). The latency of inactivation was always significantly longer for fCS stimuli as compared to fCO stimulation for the pooled data, but also for the individual animals. This difference between the two motor responses is also obvious from generating averaged PST histograms of the extensor motoneuron activity during fCO and fCS stimulation (Fig.1B(i) and 1B(ii)). Intracellular recordings from two FETi and two SETi
motoneurons substantiated the observations (Fig. 1C, i and ii respectively). The differences in the time course of the motor response became apparent from comparing the time course in membrane potential of intracellular recordings from FETi motoneurons for the different stimulus regimes (Fig.1C,D). fCO stimulation alone led to a much faster and more pronounced hyperpolarization of the extensor motoneurons as compared to fCS stimulation. In some cases, a rebound-like activation occurred in extensor motoneurons after the fCO induced inhibition (cf. also Bässler, 1988). However, this is not reflected in the PST histograms in Fig.1 due to the variability in the timing of the inhibition and resumption of motoneuron activity (see below).

Simultaneous stimulation of fCS and fCO also terminated extensor motoneuron activity (Fig.2Ai,ii). The interesting observation was that, when the fCO and fCS were simultaneously stimulated, the response of the extensor motoneurons always resembled the “active reaction”. That is, an early and fast termination of extensor motoneuron activity, reactivation of extensor motoneurons during the ongoing stimulation and a steeper hyperpolarization of the extensor motoneurons. We compared the timing of termination and reactivation of extensor motoneurons from the 7 experiments in order to confirm our impression. Interestingly, the timing for the offset of extensor activity was qualitatively and quantitatively similar to the response to fCO stimulation, when applying both stimuli together. Then, the latency for the termination of extensor activity was 0.16s (SE: 0.014s; n=58), which is significantly shorter than during fCS stimulation alone (p<0.0001), but not significantly different from the latency for fCO stimulation (p=0.11). The general decrease in latency for termination of extensor activity was even more obvious from the frequency histogram for the latency of extensor shutoff (Fig.2B). It clearly shows that with concurrent stimulation of fCS and fCO, the latency of extensor shutoff is decreased compared to fCS stimulation only. On the other hand, the latency of extensor activity recommencement during co-stimulation of fCO and fCS was
0.45s (SE: 0.02; N=7; n=58), which was significantly shorter than the latency of extensor activity recommencement with fCS stimulation (0.54s; SE:0.03; n=48; p=0.009) but significantly longer than the latency of extensor activity recommencement. However, still recommencement of extensor activity occurred during the ongoing fCO and fCS stimulation as typical for the “active reaction” (Fig.2). As seen with fCO stimulation alone, in some cases a rebound-like activation occurred in extensor motoneurons upon resuming activity (Fig.2), although this did not occur in all trials. Our data show that when simultaneous stimulation of fCO and fCS produced an inhibition of extensor motoneurons, the overall reflex motor pattern in extensor motoneurons was characteristic of the “active reaction”. This also became apparent from comparing the time course in membrane potential of intracellular recordings from FETi motoneurons for the different stimulus regimes (Fig.3A,B). Simultaneous stimulation of fCS and fCO led to a steep hyperpolarization which was comparable with the time course of the hyperpolarization caused by only fCO stimulation (Fig.3B). This was also seen in SETi recordings (Fig.3C).

The interesting question arising from the data presented above is whether the probability of occurrence of the “active reaction” was altered when fCS was stimulated at the same time as the fCO. Active reactions were evoked by flexion signals from the fCO in 32% of the trials (n=92). Stimulation of fCS does not elicit an active reaction, but does however result in inactivation of extensor tibiae motoneurons in 68% of the trials in all experiments (n=75). Simultaneous stimulation of fCS and fCO initiated an active reaction with a probability of 68% (n=85) which is identical to the probability of extensor inhibition produced when the fCS was stimulated alone. This demonstrates that if the fCO is stimulated along with the fCS, the probability of eliciting an “active reaction” increases significantly (p<0.0001).
Discussion

Our results show that the time course of extensor inactivation produced by simultaneous flexion (fCO) and load (fCS) signals from the leg is similar to the motor response produced by flexion (fCO) signals only. With simultaneous stimulation of both fCO and fCS, the latency of the extensor inhibition was significantly shorter than when fCS were stimulated alone and similar to the short latency inhibition measured when the fCO elicited an “active reaction”. Furthermore extensor activity showed the two parted motor response typical for the “active reaction” with extensor activity recommencing during ongoing fCO stimulation. Similarly, the rate of change of the membrane potential in extensor motoneurons during stimulation resembled the “active reaction” caused by fCO stimulation only. Thus, our data demonstrate that when simultaneous fCO and fCS stimulation produced a motor response, the resulting extensor and flexor activity was always the “active reaction” motor pattern. In contrast, re-activation of extensor motoneuron activity upon simultaneous stimulation of both fCO and fCS was shifted to longer latencies as compared to fCO stimulation only. These results differ from those previously reported by Bässler (1988), who reported that simultaneous electrical stimulation of axons of load sensors on the trochanter decreased the frequency of occurrence of active reactions with mechanical stimulation of the fCO. However, the results from that study are difficult to compare with our results. First, it was recently shown that load signals from the fCS, but not from the trochanteral campaniform sensilla (trCS), have a significant influence on patterning tibial motoneuron activity (Akay et al. 2001). Second, in Bässler’s study, leg afferents from the trCS were stimulated electrically together with axons of a trochanteral movement sensor, the trochanteral hair plate, in a simultaneous and unspecific manner (see his discussion). This gives rise to the concern that the observations in the study by Bässler arose from activating trochanteral hair plate afferents, rather than the trCS. In our study, we stimulated fCS exclusively using a mechanical device and combined this stimulation with a mechanical stimulation of the fCO.
In addition, simultaneous fCS and fCO stimulation increased the frequency of occurrence of the short latency inhibition in extensor motoneurons and their subsequent reactivation during simulated fCO flexion that is characteristic of the “active reaction” from 34% to 68%. This suggests that the motor response generated upon stimulation of both sense organs is not simply the result of a parallel processing of load and movement signals from the leg onto tibial motoneurons that adds up on their membrane potential. Instead, our data indicate that load signals from the fCS prime the pathways that process movement related sensory feedback in the FT-control system so as to promote the generation of the “active reaction” motor response induced by flexion signals. This does not mean that when the probabilities for the “active reaction” are increased by stimulating the fCS along with the fCO the fCS-influence on the extensor motoneurons would be abolished. Their persistent influence becomes obvious from the longer latency in recommencement of extensor activity from 0.32s to 0.45s.

What are the possible functional consequences of this interaction? When the locomotor system of the stick insect is active, sensory inputs from the fCO that signal flexion of the FT-joint terminate ongoing extensor motoneuron activity and initiate flexor motoneuron activity. This is the most prominent aspect of the “active reaction” and represents a reflex reversal of flexion signals from the fCO (Bässler, 1988). The second part of the “active reaction” is the termination of the flexor activity and the re-activation of the extensor motoneurons during increasing flexion of the FT-joint. The “active reaction” is a functional module of the walking pattern generator in the stick insect for the generation of stance phase activity. This is manifested by inhibiting extensor and promoting flexor tibiae activity and the subsequent transition to the swing phase, when extensor activity resumes (summary in Bässler, 1993). Our results suggest that load signals promote the generation of the “active reaction” and
thereby assist the generation of the stance phase motor output. During stance phase the leg carries the weight of the body and generates ground reaction forces to propel the animal that results in activation of the fCS (Akay et al. 2001; Bässler 1977). Load signals from the leg thus appear to ensure and promote the generation of movement-related reinforcement of stance phase motor output in the femur-tibia joint and in doing so they set the motor control system of the stick insect leg to stance phase mode of operation.

One possible mechanism underlying this interaction of load and movement signals is that fCS inputs to the network governing the FT-intrajoint reflex system (Sauer et al., 1996) increase the efficacy of sensori-motor pathways underlying the generation of a reflex reversal. Previous investigations have shown that during the generation of the reflex reversal, the balance of synaptic inputs to identified premotor interneurons in the FT-joint control network are shifted from excitatory to inhibitory and vice versa so that the overall motor output of the system is reversed (Bässler & Büschges, 1990; Driesang & Büschges, 1996). The other possible explanation is that as fCS signals have been shown to provide inputs to the central rhythm generating network (CRG) controlling the FT-joint locomotor activity (Akay et al. 2001) these inputs could increase the probability of generating an active reaction. Furthermore, evidence has been presented in the locust that the reflex reversal of flexion signals from the fCO is generated by a contribution of the central rhythm generating network for the FT-joint (Knop et al. 2001). On the basis of these findings it appears conceivable that fCS signals occurring in concert with movement signals from the fCO could facilitate the influence of fCO signals on the FT-joint CRG and thereby promote the generation of the reflex reversal motor output. Given the fact that in other walking systems, like the cat and the cockroach, movement and load feedback are known to contribute to the control of the motor output during stance (reviews in Pearson, 2000; Zill et al. 2004), a similar situation might exist, but at present, no data are available in these systems. Subsequent investigations in the
stick insect leg muscle control system will address which network interactions in the leg muscle control system underlie the interaction of load and movement related feedback.

Acknowledgments

We thank Drs. R.A. DiCaprio, M. Gruhn, D. McVea, K.G. Pearson and J. Schmidt for their valuable comments in the course of the work. We thank R.A. DiCaprio and S. Meyen-Southard for her help in language and style editing the paper. The work was supported by a grant of the DFG to AB (Bu857-6).
References


Figure legends

Figure 1

A: Responses of tibial extensor motoneurons to stimulation of either fCO or fCS. A(i): Extracellular recording of the extensor nerve (F2) from an experiment in which an “active reaction” was elicited by flexion signals from the fCO. On top of the recordings instantaneous frequencies are plotted separately for the FETi and SETi. A(ii): Extracellular recording of the extensor nerve (F2) from an experiment in which extensor motoneuron activity was inhibited by fCS stimulation. Dashed lines here and below mark the last extensor spike upon termination of activity and stippled lines mark the first extensor spike upon recommencement of extensor activity. B: Quantitative description of time course of activity in extensor motoneurons upon fCO and fCS stimulation B(i): PST histogram of the extensor motoneuron activity (including both SETi and FETi activity) elicited by fCO stimulation (n=29) in seven insects. B(ii): PST histogram of the extensor motoneuron activity (including both SETi and FETi activity) during fCS stimulation (n=52) in seven insects. C(i): Original recordings including extracellular recording from the extensor nerve (nl3) and intracellular recording from the FETi during fCO stimulation. Please note that during fCO relaxation no reversal is found (see also Bässler, 1988). C(ii): Original recordings including extracellular recording from the extensor nerve (nl3) and intracellular recording from the FETi during fCS stimulation. D: Comparison of average time course in membrane potentials in FETi during exclusive fCS stimulation (n=7) and fCO stimulation (n=4). The action potentials were removed by peak detection and substitution with straight line (Gabriel et al., 2003) before averaging individual. Arrowhead denotes the start of stimulation.

Figure 2

A: Responses of tibial extensor motoneurons to simultaneous stimulation of fCO and fCS. (i): Extracellular recording of the extensor nerve (F2) from an experiment in which fCO and fCS were stimulated simultaneously (same exp. as in Fig.1C). (ii) PST histogram of the average extensor motoneuron (including both SETi and FETi activity) activity during simultaneous fCO and fCS stimulation (n=58) from seven animals. B: Frequency histograms (BIN width: 50ms) comparing the latencies between stimulation onset and termination the extensor motoneuron (SETi or FETi) activity for fCO stimulation only with simultaneous fCS and fCO
stimulation (top) and for fCS stimulation only with simultaneous fCS and fCO stimulation (bottom) in one experiment.

**Figure 3**
Comparison of average time course in membrane potentials in FETi during exclusive fCS stimulation (n=7, grey trace) and simultaneous fCS and fCO (n=5, black) stimulation (A) and during exclusive fCO stimulation (n=4, grey) and simultaneous fCS and fCO stimulation (n=5, black) (B). The action potentials were removed by peak detection and substitution with straight line (Gabriel et al., 2003) before averaging. Arrowheads denote the start of stimulation. C: Comparison of the time course in membrane potentials in SETi during (i) fCS, (ii) fCO) and (iii) simultaneous fCO and fCS stimulation (spikes are truncated).
Figure 1 A: Responses of tibial extensor motoneurons to stimulation of either fCO or fCS. A(i): Extracellular recording of the extensor nerve (F2) from an experiment in which an active reaction was elicited by flexion signals from the fCO. On top of the recordings instantaneous frequencies are plotted separately for the FETi and SETi. A(ii): Extracellular recording of the extensor nerve (F2) from an experiment in which extensor motoneuron activity was inhibited by fCS stimulation. Dashed lines here and below mark the last extensor spike upon termination of activity and stippled lines mark the first extensor spike upon recommencement of extensor activity. B: Quantitative description of time course of activity in extensor motoneurons upon fCO and fCS stimulation B(i): PST histogram of the extensor motoneuron activity (including both SETi and FETi activity) elicited by fCO stimulation (n=29) in seven insects. B(ii): PST histogram of the extensor motoneuron activity (including both SETi and FETi activity) during fCS stimulation (n=52) in seven insects. C(i): Original recordings including extracellular recording from the
extensor nerve (nI3) and intracellular recording from the FETi during fCO stimulation. Please note that during fCO relaxation no reversal is found (see also Bässler, 1988). C(ii): Original recordings including extracellular recording from the extensor nerve (nI3) and intracellular recording from the FETi during fCS stimulation. D: Comparison of average time course in membrane potentials in FETi during exclusive fCS stimulation (n=7) and fCO stimulation (n=4). The action potentials were removed by peak detection and substitution with straight line (Gabriel et al., 2003) before averaging individual. Arrowhead denotes the start of stimulation.
Figure 2 A: Responses of tibial extensor motoneurons to simultaneous stimulation of fCO and fCS. (i): Extracellular recording of the extensor nerve (F2) from an experiment in which fCO and fCS were stimulated simultaneously (same exp. as in Fig.1C). (ii) PST histogram of the average extensor motoneuron (including both SETi and FETi activity) activity during simultaneous fCO and fCS stimulation (n=58) from seven animals. B: Frequency histograms (BIN width: 50ms) comparing the latencies between stimulation onset and termination the extensor motoneuron (SETi or FETi) activity for fCO stimulation only with simultaneous fCS and fCO stimulation (top) and for fCS stimulation only with simultaneous fCS and fCO stimulation (bottom) in one experiment.
Figure 3 Comparison of average time course in membrane potentials in FETi during exclusive fCS stimulation (n=7, grey trace) and simultaneous fCS and fCO (n=5, black) stimulation (A) and during exclusive fCO stimulation (n=4, grey) and simultaneous fCS and fCO stimulation (n=5, black) (B). The action potentials were removed by peak detection and substitution with straight line (Gabriel et al., 2003) before averaging. Arrowheads denote the start of stimulation. C: Comparison of the time course in membrane potentials in SETi during (i) fCS, (ii) fCO and (iii) simultaneous fCO and fCS stimulation (spikes are truncated).